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FORMALIN IN THE ZOÖLOGICAL AND HISTOLOGICAL LABORATORY.

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How often in the memory of most of us has the suffering world been startled by the announcement, often by the highest authority, of some wonderful medicine, specific or elixir. These, one after another, have been put to the test and have taken their places among the useful remedies, but on a plane below, often far below, the first claim and the initial hope. Is it to be thus with Formaldehyde as antiseptic, as remedial agent, as preservative, as a histological fixative? It can only find its true plane among its kind by conscientious trials made and reported by investigators. During the last year it has been used in the laboratory of the Ohio State University quite extensively as a preservative and for histological purposes. I have brought together in this paper the results and the reflections to which they obviously lead. Papers have appeared in the last year or two giving results from laboratories along similar lines. I may say at the outset that my experience leads to conclusions essentially in accord with results heretofore published.

In the statement of trials I shall by "per cent." mean per cent. by volume of the forty per cent. solution of formaldehyde in water, procured of Scharing & Glatz of New York, and distilled water. I have found this standard solution known as formalin fairly constant, and so a reliable solution may be thus quickly made of pretty constant strength.

I. As a preservative I have tried it on a variety of objects, using varying strengths with results to be noted.

Experiment 1. Small sunfish perfectly fresh was put into two per cent. formalin Feb. 2; March 1 it was well hardened, without shrinkage; colors fairly preserved compared with the best alcoholic

specimens; eyes better; fins extended. The fluid was filtered, the bottle sealed and given a place on the shelf. July 1, no perceptible change observed.

Experiment 2. Two common eels from the market were immersed in four per cent. March 11. July 1, examination showed that they were in perfect condition as to color, absence of shrinkage and firmness. The fluid had colored slightly and there was a sediment. Filtering rendered the specimens fairly representative museum preparations, fit for either systematic or anatomical purposes.

Similar trials were made with a variety of fish naked and scaled, large and small, with uniformly good results. A stronger solution, up to ten per cent., was found superior for larger specimens; the colors are better preserved in solutions as strong or stronger than three to four per cent.

Experiment 3. *Amblystoma jeffersonianum* killed with chloroform March 1, put immediately into two per cent. formalin; at the end of four days it was found to be quite hard, color very slightly changed, costal grooves plainer than in the living animal. The fluid was filtered and was still clear July 1, while the preparation left nothing to be desired.

Experiment 4. *Amblystoma tigrinum* treated similarly but put into four per cent. gave excellent results. So far as appears, for museum, systematic or anatomical, purposes it was no better than specimen in experiment 3.

Experiment 5. *Amblystoma tigrinum* was killed April 5, with chloroform and the mucus washed off with water as in experiments 3 and 4; it was then placed in equal parts of ninety-five per cent. alcohol and two per cent. formalin. July 1 the specimen was far superior in appearance and preservation than I have ever been able to prepare by means of alcohol alone. I cannot say it is superior to that of experiment 4.

Other Amphibia and several Reptilia have been preserved in a water solution of formalin and in alcohol-water formalin with excellent results so far as the time that has elapsed permits of a demonstration. It seems to be the best method for Ophidia.

Experiment 6. A tongue of a lion was thoroughly washed in water to remove blood, mucus and dirt and treated February 5 to a five per cent. solution of formalin. July 1 it was found well hardened, in clear fluid with but slight indications of shrinkage.

A camel's tongue, larynxes of spider monkey, camel and kangaroo were cleaned, washed and preserved in from two to four per cent. during February and March; they are at the present time fine preparations.

Experiment 7. Several trials were made to harden and preserve brains, with different per cents. of formalin, with alcohol and formalin, and with formalin and bichromate.

I will speak particularly of a few :

1. A brain of spider monkey removed twenty-four hours after death was placed in four per cent. formalin. In a very few days it was firm, remained perfectly white and did not noticeably shrink. July 1, it was all that could be desired in a museum specimen.

2. The brain of a kangaroo was hardened in forty or fifty per cent. alcohol and two per cent. formalin, equal parts; the results were no better; the specimen was as good and prepared in less time than by alcohol alone; and of course much more cheaply prepared.

3. Brains of dogs and cats were prepared for anatomical purposes by adding to the usual bichromate solutions one or two per cent. formalin, a smaller quantity of fluid was necessary, not so many changes and a great gain in time; the material was excellent, not brittle and with fine contrasts.

Before stating my general conclusions as regards this agent in preserving museum and anatomical preparations, I would like to add a note as to its usefulness in another direction, viz., in preserving animals for dissection. If the subject is a small dog, its blood vessels are washed out with salt solution and 250 cc. more or less of five per cent. formalin injected. Thus prepared and kept in a cool place, when not under examination, it may be used by the dissector day after day and for several weeks without unpleasantness. The brains of animals thus treated may be removed for study much longer after death than in any

other way known to me, at the same cost of material and time.

The advantages of formalin in the lines discussed above may be summarized briefly thus: It is cheaper than any other method that gives good results; it gives results in much less time; the colors are better preserved and there is less change of form by shrinkage or by swelling; its penetrating power is excellent so that insects, crustacea, etc., preserved in it are fit for work on the internal organs; for mollusks and vermes it is also excellent.

Its disadvantages should likewise be stated. It is extremely volatile and the jars have to be sealed with care. I have not had it in use long enough to decide how great an obstacle this will prove in the museum. Again, the water solution will freeze and not all museums are at all times above 0°C. In such cases, if not in all, hardening in formalin and transferring to the lowest per cent. alcohol that will preserve would seem to be the better and cheaper way. I have not yet had an opportunity to demonstrate to what extent preparations thus preserved may be treated to additional alcohol to replace that lost by evaporation, without a precipitate.

II Experiments with formalin for histological purposes were undertaken in February last. The visceral organs of *Cryptobranchus* were placed while perfectly fresh in a suitable dilution of borax carmine with five per cent. formalin; the third day the pieces were washed, the stain fixed and dehydration in sixty per cent. alcohol commenced; they were passed rapidly through alcohols of increasing strength, and at the end of seven to ten days were infiltrated with paraffin and ready to cut. The liver, intestines and kidney were sectioned, leaving nothing to be desired in stain or character of the cells.

Parts were at the same time hardened in five per cent. without bulk stain, also in dilute alcohol and formalin. We concluded that the rapidity and less shrinkage were alone sufficient to recommend the use of the new agent.

Mr. J. H. McGregor, who was conducting an investigation of the central nervous system of *Cryptobranchus* in the same laboratory, used the agent in combination with alcohol and other

agents to his entire satisfaction. He will doubtless give his results when his paper is printed. Another student used it successfully in preparing the eye.

I may say, in short, that the result of the few months of trial warrant a high estimate of it for fixing animal tissues.